Review

The Immunology of Onchocerciasis

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Onchocerciasis is a chronic parasitic disease caused by the filarial nematode *Onchocerca volvulus* and transmitted by insects (Diptera: Simulidae) of the genus *Simulium*.[1,2] It is endemic in intertropical regions, having been found in most African countries, in Saudi Arabia and North Yemen, and in six Latin American countries (Brazil, Colombia, Ecuador, Guatemala, Mexico, and Venezuela).[3,4] It is estimated that 20–40 million people are affected with onchocerciasis resulting in 250 to 400 thousand cases of blindness.[4,5] Although this disease does not directly affect mortality, it causes extreme itching and incapacitating eye lesions and may be the precipitating factor in reduced social productivity and mass abandonment of agriculturally fertile regions where the population is concentrated.[4,6]

Onchocerciasis is a disease with focal distribution, predominating in areas adjacent to fast-flowing water where the insect vector breeds.[4] Control of the disease is most commonly through the use of larvacides, such as DDT and abate, on the vector population of simuliids. This strategy, used by the World Health Organization since 1974 in the Upper Volta river basin, is not always practical due to the high costs involved in covering vast areas and in maintaining this type of control for a period of at least 20 years.[4] Direct treatment of patients through chemotherapy as a means of halting transmission is also impractical, because the drugs now available for treatment, diethylcarbamazine (DEC) and suramin, may produce severe side effects, requiring strict medical supervision usually unavailable in endemic regions.[6] This situation, in addition to the many observations that suggest the participation of immune mechanisms in the majority of characteristic onchocercal lesions, has led in the last decade to the intensification of immunologic studies of the disease. This review summarizes the most important of these studies, particularly the progress made in knowledge of the agent's antigenicity, exploration of the host's immune response, understanding the immunopathology of the disease, and perfection of the immunodiagnosis of onchocerciasis.

Parasite Antigenicity

At present, the genus *Onchocerca* is considered a modern taxon that is still in a rather rapid process of speciation.[7,8] Instability in the species *O. volvulus* is shown with the appearance of new strains, demes, or subspecies as a consequence of adaptation to different ecosystems and evolution in distinct vectors. These biologic variants are differentiated by their morphobiometry,[9,10] their enzymatic patterns,[11,12] their pathogenicity in chimpanzees[13] and rabbits,[14,15] their intermediate hosts,[16,17] and their antigenic composition.[18] Through the study of these characteristics, it has been determined that African onchocercal strains are different from those found in the Americas and that within the African continent itself the forest and savannah demes are significantly different.[17,18] The life cycle of *O. volvulus* takes place in blood-sucking females of various anthropophilic species of the genus *Simulium*[3] and in its vertebrate host, man. In man, the infective helminth larvae, introduced by the vector during its ingestion of blood, migrate to various sites in the subcutaneous cellular tissue. Then, through a little-understood development, they mature into adults of both sexes. These adults are progressively enveloped in host collagenous tissue, resulting in characteristic subcutaneous nodules (onchocerco-

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mas).[19,20] Reproduction takes place within the onchocercomas, where the females, on completion of the gestation period, release microfilariae that enter the lymphatic system. These microfilariae invade the tissues, becoming localized in the eyes and skin, although they may also be found in lymphatic ganglia,[21] the blood,[22] and the urine.[23] Infection of female simuliids takes place when they feed on an individual with microfilariae in the skin. A variable number of parasites are sucked up with the blood, the majority of them into the simuliid's stomach, where they become enclosed in the peritrophic membrane and die. The remaining microfilariae pass to the thorax of the insect, where during the next 6–9 days they pass through two stages in their metamorphosis to become infective larvae designated as larval stage 3 (L_3).[24] The L_3 larvae then move to the mouth apparatus of the simuliid and are released into the human host the next time the fly bites. The time for maturation of these larvae into adults within the human host is 3–18 months; microfilariae in the host are not detected during this prepatent period. In each one of the stages of the life cycle of *O. volvulus,* numerous antigenic substances are produced by the helminth. Many of these are potent immunogens that stimulate the production of antibodies in naturally infected individuals as well as in experimentally immunized animals.[25]

The identification, the physicochemical characterization, the isolation, and the study of the part that these immunogens play in the host–parasite relationship are the fundamental objectives of the immunologic study of onchocerciasis.

Adult Filarial Antigens

The antigenicity of the adult stage of *O. volvulus* has been studied by immunofluorescence techniques using the whole individual worm. In addition, soluble extracts and excretion–secretion products of the worm have been examined by electrophoresis, molecular filtration, isoelectric focusing, and immunologic analysis.

Whole Worms. The source of adult onchocercal worms has almost always been infected human beings, because it has not been possible to establish active *O. volvulus* infections in suitable laboratory animals (infections have been produced in chimpanzees,[13] but these animals are difficult to handle and their cost is prohibitive). For experimental use, fragments or sections of the adult filariae are mechanically extracted from onchocercomas then examined by indirect immunofluorescence using frozen sections[26] or slices imbedded in paraffin.[27] The results obtained from these examinations show the presence of multiple antigen determinants on the external surface and cuticle of the filariae.

Soluble Extracts. Until the introduction of the collagenase digestion technique for onchocercomas,[28] extracts were obtained from adult worms isolated by homogenization and centrifugation of nodules.[25,29] These extracts revealed the presence of immunogens of parasitic origin but were contaminated by molecular substances produced by the host. Two kinds of parasite antigens were found, those specific to *O. volvulus* and those in common with other species of parasitic helminths.[25,29,30] Among the contaminants of host origin, human albumin and IgG were identified.[29] Antigens common with other parasites as well as those from the host were important sources of unspecific reactions in immunologic tests for onchocerciasis. It is possible now to eliminate these unspecific antigens by the use of soluble extracts from worms isolated from nodules digested enzymatically. This process destroys a large part of the nodule proteins, after which purified extracts are obtained by immunoabsorption in fluid phase or by affinity chromatography.[29] The application of physicochemical and immunologic techniques to the study of soluble extracts from African strains revealed that they contain numerous antigenic macromolecules and suggested the existence of antigenic differences related to the geographic location of the deme.[18] Electrophoresis in SDS polyacrylamide gels produced 8–13 polypeptide bands; the hemagglutination test revealed that adult filariae from the Cameroon savannah had an antigen that differentiated them from the forest filarial strain in that country. If this antigenic difference between closely related demes is confirmed, it will have to be taken into account in the analysis of the host immune response as well as in the study of the role that antigen diversity plays in the pathology of onchocerciasis and its significance in relation to the evolution of *O. volvulus.*

Excretion–Secretion Products. Collagenase digestion of nodules has made it possible to obtain whole, viable filariae from surgically extracted onchocercomas. These filariae survive for several days in cultures free of human serum,[31] establishing a metabolic exchange with their environment; as a consequence, the organisms liberate excretion–secretion products that accumulate in the medium. Various authors have suggested that the excretion–secretion antigens of parasites are less complex and more specific than soluble extracts.[32,33] A preliminary study of supernatants in the culture medium of adult *O. volvulus* filariae tends to confirm this finding. The micro-ELISA test conducted with this antigen prepa-

ration revealed antibodies against the parasite in a high percentage of onchocerciasis cases and was negative in patients with other helminth infections (toxocariasis, trichinosis, strongyloidiasis, bilharziasis, and echinococcosis), which usually produce cross-reactions in the presence of crude extracts of *O. volvulus*.[31] These results indicate that the excretion-secretion products may be very useful in the diagnosis of onchocerciasis. The analysis of these products has also revealed antigenic differences between male and female adults of *O. volvulus*.[31]

Microfilarial Antigens

Since the 1960s, there has been increasing interest in using microfilarial antigens in the immunologic study of onchocerciasis.[18,34-37] As with adult filariae, distinct preparations, ranging from the whole microfilaria to its excretion-secretion products, have been examined.

Whole Microfilarae. Microfilariae have been used as antigens in immunofluorescence tests.[34] Through this procedure it has been demonstrated that the microfilariae possess surface antigens that interact with antibodies present in the serum of patients with onchocerciasis. This technique has also permitted the discovery that microfilariae obtained from the skin have IgG adhering to their surface while intrauterine microfilariae do not.[34,38]

Soluble Extracts. Soluble extracts have been most often prepared from microfilariae dissected from adult worms isolated from onchocercomas[35,36] and disrupted either mechanically[36] or by ultrasonification.[35] Analysis of antigenicity is customarily through protein separation,[18] immunoprecipitation,[35] hemagglutinarion,[18] and intradermic tests.[35,36] Electrophoresis studies in SDS polyacrylamide gels reveal a lesser number of polypeptide bands when compared with soluble extracts from adults, but they demonstrate also that the larger bands are habitually in the same position in both stages.[18] Soluble microfilarial antigens have been shown to have low reactivity in immunoprecipitation[35] and haemagglutination[18] tests but that they show high sensitivity (85-86%) and acceptable specificity (7-13%) in intradermic tests.[35,36]

Excretion-Secretion Products. Beginning in 1979, methods allowing the *in vitro* culture of *O. volvulus* microfilariae became available. At that time, a procedure was described that utilized a biphasic medium containing rabbit red blood cells, human serum, and nutrient agar.[9] Microfilariae survived in this medium for prolonged periods and liberated into it excretion-secretion products with antigenic properties; however, immunochemical analysis of these products was made difficult due to the presence of macromolecules of rabbit blood and of human serum proteins in the medium and on the surface of the microfilariae. It was later discovered that microfilariae could also survive in chemically defined media,[37] and a simple and practical technique for the selective recovery of nodule microfilariae was described.[39] This latter procedure permitted the demonstration that both pH and temperature were very important factors in aiding the survival of *in vitro* microfilariae and revealed that the subculture of larvae reduced the contamination of excretion-secretion products by human serum proteins, with minimal values being reached after nine subcultures carried out during a period of 96 hours. The immunochemical analysis of the excretion-secretion products from *O. volvulus* has demonstrated that they generate 20-30 polypeptide bands in SDS polyacrylamide gel electrophoresis revealed by autoradiography.[40] After absorption with immunosera against human plasma proteins, the number of bands was reduced to 10, showing three major and seven minor components whose molecular weights varied between 10,000 and 125,000 daltons.[40] Various of these components were immunogenic, and four of them produced characteristic bands in immunoprecipitation tests. The crude supernatant of the culture medium did not contain antigens in common with crude soluble extracts from other nematodes (*Loa loa, Onchocera gutturosa, Litomosoides carinii, Dirofiliari immitis,* and *Ascaris lumbricoides*) and was free of hepatitis B virus.[40]

Invective Larvae Antigens

Detailed reports of the antigen composition of onchocercal L_3 larvae have not yet been published, but *in vitro* studies have revealed eosinophils adhering to larvae of this stage. These larvae have been shown to be highly immunogenic, showing strong cuticular interactions with antibodies present in the sera of patients with onchocerciasis.[41]

Vector Antigenicity

As the simuliids ingest blood, they inoculate the host with several macromolecular substances from their salivary glands and the digestive tube.[42] Some of these substances behave as antigens, provoking immediate hypersensitivity reactions (local and systematic) as well as the production of different types of antibodies.[43,44] This response may give rise to bronchospasm and dermatitis,[43] cutaneous eruptions,[45] and hemorragic syndromes,[46,47] and probably contributes to the pathogenesis of some of the manifestations of onchocerciasis. Additionally, vector an-

tigens may be associated with surface molecules of *O. volvulus* L_3 larvae, intervening in the initial antigenic stimulus caused by the parasite.

Host Immune Response

The majority of persons resident in endemic onchocercal areas do not present clinical manifestations of this infection, even when parasitologic examinations of the skin reveal the presence of microfilariae.[44] Among the infections with clinical manifestations, two principal forms are recognized: localized, which is limited to one of the extremities and is characterized by itching, darkening, and thickening of the skin;[48] and generalized, with symptoms that include disseminated itching, different types of dermatitis, subcutaneous nodules, lymphedema, lymphoadenopathy, ocular lesions, and lesions in other visceral tissues.[49,50]

In the localized form, a parasitologic examination of the skin may be negative or reveal very few microfilariae, and a histologic examination may show acanthosis, hyperkeratosis, extensive inflammatory infiltrates with numerous plasmacytic cells, few lymphocytes, and a reduced number of histiocytes.[51] In the generalized form, there are abundant microfilariae in the skin and the histologic findings are variable, although there is an absence of inflammatory lesions of granulomatous reactions around the microfilariae living in the skin and the eyes.[41] This reveals the existence of a spectrum of clinical-pathologic manifestations, similar to those observed in leishmaniasis and leprosy, the reasons for which are still unknown. Some authors feel that this diversity of manifestations may be due to the variation in pathogenicity detected in distinct *strains of onchocercal infective larvae*.[14,18] It has been suggested recently that this clinical spectrum may be the consequence of a heterogeneous immune response on the part of the host.[38,51] It is still not known precisely what part cellular mechanisms and antibodies play in the pathogenesis of onchocerciasis, but information obtained during the last decade had confirmed the existence of different immune response patterns in the distinct clinical-pathologic types of this infection.

T-Lymphocyte-mediated Immune Response

The T-lymphocyte-mediated immune response has been studied through intradermic tests made with homologous antigens (from different stages of *O. volvulus*) and with heterologous antigens (from other nematodes, in particular *Necator* and *Ascaris*), as well as through techniques that reveal, *in vitro*, different products from activation of the lymphocytes.[49,51,52] These studies have demonstrated that delayed hypersensitivity (Type IV of Coombs and Gell[53]) is associated with the localized form of the infection, being generally absent in the generalized form of onchocerciasis. It has been postulated that the depression of the T-lymphocyte-mediated immune response could be due to the presence of circulating immune complexes that inhibit the effective functioning of T cells, to the existence of suppressing B cells, or to the action of parasite substances that induce depression of cellular activity.[51] Recently, our group demonstrated that precultured peripheral lymphocytes from patients with depression of delayed cutaneous hypersensitivity proliferated normally when they were stimulated with phytohemagglutinin, in the presence of normal human serum.[52] When the stimulation was made in the presence of autologous sera, however, inhibition was observed in 58% and stimulation in 26% of the cases, with 16% of the cases being unaffected.

In addition, in 78% of these patients depression of the proliferative response of precultured lymphocytes was shown when they were stimulated with alloantigens in the presence of autologous serum. Finally, the sera of five patients from the same group produced allogenic inhibition in mixed cultures of lymphocytes from normal and ill persons.[52]

These observations suggest that in patients with severe generalized forms of onchocerciasis there exist serum factors (eg, antigens, antibodies, or immune complexes) that block the function of T-lymphocytes at either the central or peripheral level. This is translated into a depression of delayed hypersensitivity (type IV of Coombs and Gell), which is manifested by negative late intradermic tests and by hyporesponsiveness of lymphocytes cultivated *in vitro* after exposure to homologous antigens, alloantigens, or mitogens. The recuperation of this reactivity after the preculture indicates that the depression is acquired and suggests that it is due to blockage of the surface receptors of the T-lymphocytes.

Polymorphonuclear-mediated Immune Serum-dependent Cytotoxicity

A reproducible and standardized procedure of microfilarial cryopreservation has permitted the demonstration that eosinophils and neutrophils from normal individuals have the capacity to kill microfilariae of *O. volvulus*.[54] The addition of fresh serum increases the lethal capacity of the polymorphonuclear cells and especially of eosinophil-mediated cytotoxicity, which suggests that the complement system must play an important part in the lysis of the parasites.[54] In the same study it was demonstrated that microfi-

lariae derived from cryopreserved onchocercomas were not capable of activating complement directly, whereas derivatives from the skin appeared to be able to do so without a necessity for antibodies. This supports the existence of differences in the surface structure of intrauterine microfilariae compared with those from the skin,[34,38] thus enabling an explanation for the presence of surface immunoglobulins on microfilariae from the skin. Similar results were obtained in experiments made with fresh microfilariae and with cells from infected individuals.[54] These experiments indicate that there are mechanisms that assure the host of some resistance against onchocercal infection; however, we lack *in vivo* evidence of this resistance, especially in consideration of the fact that this cytotoxic mechanism could be blocked by some factor that impedes the interaction of the cell-surface receptors with the microorganisms, and also that the different components of the reaction taking place *in vitro* may not have access to each other *in vivo.*[54]

B-lymphocyte-mediated Immune Response

Infection with *O. volvulus* stimulates the synthesis of antibodies directed against antigenic determinants of the different stages found in the human host (microfilariae, L_3 larvae, and adults). These antibodies belong fundamentally to the IgG, IgM, and IgE classes of immunoglobulins.[38,55] The serum concentrations of these antibodies are greater in patients with the severe, generalized form of the infection than in those with the milder, localized form.[38] In endemic zones it has been observed that serum levels of IgG and IgM are positively correlated with the parasite load and with microfilaruria.[23] The presence of immunoconglutinins also has been reported, suggesting that these antibodies, directed against certain antigens or components of the complement system, may contribute to the pathogenesis of onchocerciasis.[38] Greater concentrations of immunoconglutinins were detected in patients with the localized form of onchocerciasis, this being associated with a reduced parasite load and with an intense lymphocyte reaction to phytohemagglutinin *in vitro.*[38] Based on observations that show the participation of immunoconglutinins in clearing circulating immune complexes, it has been suggested that these antibodies may increase the resistance to infection with *O. volvulus.*[38]

Immunopathology

The human immune response to *O. volvulus* appears to be the principal cause of the majority of the characteristic onchocercal lesions.[41] Hypersensitivity reactions are apparently directed against the dead microfilariae (natural or due to chemotherapeutic action.[41,56] On the other hand, the depression of the T-cell-mediated response, which appears in the severe, generalized form of the disease,[49,51] seems to be one of the causes of the infection's chronic nature and of the degenerative lesions that are due to the prolonged presence of the parasite in the host. Recent work suggests that this immunodepression may be due to serum factors, among which antigens and circulating immune complexes have been detected.[57-60]

Immediate Hypersensitivity (Type I of Coomb and Gell)

In onchocerciasis, the immunologic fight begins at the moment the infective larvae penetrate the skin, having been introduced by the vector. These larvae possess inherent surface antigenic determinants and are also likely to carry immunogens of simuliid origin. It is possible that such antigens stimulate the synthesis of diverse types of antibodies, among them some belonging to the IgE group. These antibodies, while interacting with new doses of antigens, may be responsible for some of the immediate cutaneous hypersensitivity reactions observed in endemic areas only minutes after the individual has been bitten by the simuliid.[42,45]

Once the adult worm has developed in the subcutaneous tissue and the females have become fertilized, microfilariae appear in the tissues. Larvae of this stage are implicated in causing the cutaneous and ocular lesions of this disease.[41] According to the most accepted hypothesis,[56] these lesions are the result of inflammation provoked by immediate hypersensitivity reactions due to the death of the microfilariae in the patient. Microfilarial death brings about the liberation of antigenic determinants that were hidden, which then react with preformed IgE antibodies. Interaction with these antibodies provokes mastocyte degranulation, the liberation of vasoactive amines, anaphylaxis (local or systemic), and inflammation.[56] The constant repetition of this reaction over time produces the functional and anatomic alterations observed in the skin and eyes of patients with onchocerciasis.[41] The same phenomenon constitutes the physiopathologic base of the accidents, varying in severity, that occur after treatment with DEC.[56,61] This drug promotes *in vivo* the mobilization and the death of *O. volvulus* microfilariae, causing anaphylactic reactions in the majority of patients, generally localized in the skin and eyes (Mazzotti's reaction). In individuals with elevated parasite loads, the reaction becomes generalized and may be fatal.[61] Localized

reactions begin between 15 minutes and 12 hours after the administration of 50–100 mg of DEC. Reactions generally begin with intensification of preexisting symptoms (pruritus of the skin and eyes, eye pain, photophobia and lacrimation, and localized inflammation [cutaneous and conjunctival edema]). This symptomology may be followed by a rash or generalized dermatitis accompanied by a burning sensation in the skin. The lymphatic ganglia that drain affected cutaneous areas become swollen, tender, and painful. Those patients with microfilariae in the anterior part of the eye suffer from conjunctival hyperemia and photophobia; occasionally, acute iridociclitis develops. Lesions also occur in the posterior section of the eye, which, although they are not immediately evident through clinical examination, may be demonstrated by fluorescent angiography. The generalized reaction has a similar beginning but is accompanied by systemic disturbances (chills, restlessness, sweating, cough, and syncope). Additionally, the patient may present fever, tachypnoea, tachycardia, hypotension, and hematologic changes with eosinophilia, neutrophilia, lymphocytopenia, and monocytopenia. These types of reactions are responsible for the fatal accidents, of which various cases have been reported in the literature.[61]

Hypersensitivity Mediated by Immune Complexes (Coombs and Gell Type III)

Hypersensitivity caused by immune complexes of the local inflammatory form (Arthus type) has been observed in onchocerciasis.[51] This phenomenon becomes evident about 6 hours after the injection of *O. volvulus* antigens and disappears within 24–36 hours. It is characterized clinically by diffuse infiltration surrounded by erythema and histologically by edema and cellular infiltrates in which polymorphonucleii predominate.[51] The generalized form, comparable to serum sickness, has not been documented reliably, but it has been suggested that it may contribute to the pathology of various of the lesions observed in this disease, such as retinal vasculitis, optic atrophy, uveitis, and perivascular polymorphonuclear infiltrates.[57]

Circulating Immune Complexes

Various studies have demonstrated the presence of circulating immune complexes in patients with onchocerciasis.[57-60] Their prevalence and concentration in the serum varies in relation to the technique used to detect them. They contain immunoglobulins of groups IgG, IgM, and IgE.[57] It has not been possible to demonstrate the presence of *O. volvulus* antigens in them, and they do not show a correlation either with the parasite load or with the titer of specific antibodies. It has been postulated that the circulating immune complexes fulfill the function of immunomodulators during the course of the disease and that their interaction with different host lymphocyte populations (suppressor and effector cells) may cause the immunodepression observed in the severe generalized form of the infection.[60,62]

Circulating Antigens

Recently, it has been observed that the serum of patients with onchocerciasis may contain soluble antigens of *O. volvulus.*[63] The parasitic specificity of these antigens has been demonstrated through immunologic identity reactions between substances in the serum and immunogenic components from soluble extracts of the adult stage of *O. volvulus*. Apparently, a correlation between the number of microfilariae in the skin and the concentration of circulating antigens does not exist. In our laboratory, we have observed that antigenemia is associated with the depression of delayed cutaneous hypersensitivity, which leads us to believe that circulating antigens, isolated or forming immune complexes, may intervene in the modulation of the T cell response, operating at the level of the surface receptors of the effector cells and/or through the stimulation of suppressor cells.[64]

Immunodiagnosis

The immunodiagnosis of onchocerciasis has been the object of numerous works and several reviews.[65,66] To this end, practically all techniques that explore the immune response have been employed. The substances used most frequently as antigens are heterologous preparations derived from helminths different than *O. volvulus,* most easily obtained in laboratories.[66] This has produced high indices of unspecificity in all tests and has greatly limited the possibility of application of immunologic techniques in the diagnosis of cases, in posttherapeutic control, and in the realization of epidemiologic surveys. The introduction of enzymatic digestion for the isolation of adult filariae,[28] cryopreservation for obtaining viable microfilariae,[9] and culture in specific media for the preparation of excretion-secretion antigens[40] has made possible the preparation of homologous antigens and the use of highly sensitive techniques, such as immunoenzymatic and radioimmune assays. This has allowed great progress in the immunodiagnosis

of onchocerciasis. It is beyond the scope of this review to make an exhaustive analysis of this theme; we have thus attempted to discuss briefly the immunodiagnostic tests for onchocerciasis that we consider most useful at this time.

Cutaneous Tests

The use of *O. volvulus* microfilarial antigens[35,36] has again made cutaneous tests useful, especially for seroepidemiologic surveys. Using crude soluble extracts, the intradermic-reaction technique gives positive results in 85-86% of patients with onchocerciasis and false-positive results in 7-13% of patients without onchocerciasis or with other helminthic infections.[35,36] The use of excretion-secretion products in the same test appears to increase the sensitivity of the test to 80-100% while maintaining a low index of unspecificity.[37] In 1981, a successful adaptation of the multiple prick test was reported, this being carried out with excretion-secretion antigens to explore cutaneous reactions in onchocerciasis.[40] This test produced positive reactions in 81% of patients with confirmed cases of the infection, whereas it produced false-positive results in 13.5% of patients with ascariasis, 4.5% of patients with loiasis, and 2.4% of individuals in a control group. These findings give hope that the intradermic-reaction and multiple prick tests with microfilarial antigens may be useful in the diagnosis of onchocerciasis, especially for epidemiologic surveys and evaluation of control programs.

Immunoprecipitation Tests

In onchocercal helminthiasis, immunoprecipitation tests have had their greatest application in the immunologic analysis of soluble extracts of *O. volvulus*.[25] The most important contribution of this technique to the immunodiagnosis of the disease involves immunoelectrophoresis, which permits the individualization of antibodies directed at specific antigens in the agent. In the hands of the scientists at the Lille school, this technique was used to individualize antibodies for diagnosis, although the antigen used in their study was *Dipetalonemia viteae*, a filaria that is easily maintained in hamsters.[66,67] Using *O. volvulus* antigens, immunoprecipitation tests revealed antibodies in 89% of patients with confirmed cases of onchocerciasis.[66] The heterologous antigens formed precipitating systems in 87% of patients.[67] In immunoelectrophoregrams obtained with homologous antigens, specific precipitation systems were observed that permitted the serum diagnosis of onchocerciasis and made possible differential diagnosis with other filarial infections.

Immunofluorescence

The use of immunofluorescence in filariasis, including onchocerciasis, has been reviewed extensively.[68] The principal advantage this method offers is the economical use of antigens such that only a few adult worms can supply sufficient material for thousands of tests. These tests may be carried out with *D. viteae*, but the use of *O. volvulus* notably increases the specificity and sensitivity of the procedure.[68] The study of sera from individuals located in hypo-, meso-, and hyperendemic areas of onchocerciasis has demonstrated that the test is able to discriminate between these levels of infection. Its use in serum-epidemiologic surveys is facilitated because it can be carried out with small quantities of blood collected in filter paper.[68]

Indirect Hemagglutination

Indirect hemagglutination, when used with *O. volvulus* antigens, is a valuable immunoepidemiologic diagnostic technique for onchocerciasis. This procedure has been successfully adapted to the study of blood samples recovered from filter paper, giving results comparable to those obtained from samples of blood extracted through venous puncture.[69] This technique has given positive results in 95% of individuals with confirmed microfilarial infections and in 82% of those persons with nodules.[70] In endemic zones, up to 22.5% of the population have positive hemagglutination tests, although they have neither nodules nor microfilariae. This is in contrast to nonendemic areas, where the technique gives only 3-3.5% positive results. The high reactivity observed in endemic areas may be due to the presence of infected individuals who are in a prepatent phase of the disease.[70] The concentrations of antibodies revealed by indirect hemagglutination rise in proportion to the parasite load; the percentages of positive reactions by age and sex vary in relation to the prevalence of the infection.[70]

Immunoenzymatic Assay

In 1975, the enzyme-linked immunosorbent assay (ELISA) was applied to the diagnosis of onchocerciasis.[71] The results were deceptive because the *O. volvulus* antigen gave very high values for optical density not only when incubated with control serum but also when incubated with phosphate-buffered

saline (PBS). It is considered that this reactivity was due to contamination of the antigen with human proteins, which reacted with the anti-immunoglobulin human-enzyme complex.[71] Later, the technique was reevaluated in a comparison with indirect immunofluorescence, using a purified homologous antigen in a study of 450 sera.[72] The diagnostic value of the test was determined using 90 sera from patients infected with *O. volvulus* and 230 sera from individuals resident in an endemic area. The percentage of positive tests (80%) in the former group was similar to that using indirect immunofluorescence, but in comparison, the immunoenzymatic assay appeared superior for diagnosis because it gave a lower positive index in the group of residents in the endemic area.[72] It was later reported that the procedure was significantly improved in specificity when excretion-secretion products were used as antigens.[31] In our laboratory, this technique was found useful in discriminating distinct levels of endemia and for the epidemiologic study of populations.

Radioimmunoassay

In recent years, the radioallergosorbent test (RAST) has been applied to the quantification of IgE antibodies in onchocerciasis.[55,73,74] Two procedures have been used, one that employs paper discs[73] and one that uses sepharose beads[55,74] as antigen supports. This procedure has confirmed the existence of IgE antibodies against *O. volvulus* in the serum of patients with onchocerciasis[74] and has shown that these antibodies have less cross-reactivity with heterologous antigens of the parasite than those belonging to the IgG group.[55] The sensitivity of RAST with soluble antigens from the adult stage of *O. volvulus* reaches 96%, a value significantly higher than that obtained using the same test with antigens from *D. viteae* (89%) or *D. immitis* (54%).[74] The relative specificity of IgG and IgE antibodies has been examined using four serum pools from patients infected with *A. lumbricoides, Brugia malayi, O. volvulus,* and *Wuchereria bancrofti*.[55] The IgE antibodies were quantified using the RAST technique, whereas the IgG antibodies were studied using *Staphylococcus* protein A radio immunoassay (Staph. A-RIA). The analysis of the binding curves in immunosorbent preparations with antigens from *A. lumbricoides, B. malayi, D. viteae,* and *O. volvulus* in RAST and Staph. A-RIA, showed less cross-reactivity in IgE.[55] These findings have renewed interest in the development and evaluation of techniques, which will quantify IgE antibodies for the purpose of incorporating them into the immunodiagnosis of onchocerciasis.

Conclusions

The study of the immune response has confirmed the clinical findings that onchocerciasis behaves as a spectral disease where localized, reactive, and autolimited forms are observed in which the immune response is preserved and in progressive, generalized forms that exhibit a frank depression in cellular immunity. The search for factors that explain these changes has led to the demonstration of the presence of antigens and circulating immune complexes in immunodepressed patients. It is thought that these substances may be causal factors in immune depression due to interaction with different lymphocyte populations. Concerning immunopathology, there is evidence that demonstrates the participation of immediate hypersensitivity in the pathology of cutaneous lesions in the disease, and it has been proven that the local and systemic reactions that appear after the administration of the drug, DEC, are the results of anaphylactic phenomena due to the interaction of microfilarial antigens with IgE antibodies.

The application of immunologic techniques and concepts to the study of onchocerciasis has provided significant advances in the understanding of certain aspects of the disease. The analysis of antigenicity of the causal agent has shown that *O. volvulus,* like other protozoan or metazoan parasites, is a physiologically and biochemically complex organism that has a great variety of antigens of low and high immunogenicity, both specific to itself and in common with other parasites. The study of the antigenic composition of the different stages of the life cycle of this nematode has revealed important differences in its antigenicity, these accompanying the physiologic and biochemic changes that occur in the parasite. The antigens of *O. volvulus* also vary between African and American strains, between forest and savannah strains in Africa, and among filariae taken from the same host. The preparation of antigenic extracts for analysis and for use in immunologic techniques has changed fundamentally in recent years. Immunoelectrophoresis with homologous antigens continues to be the only immunologic procedure capable of permitting an etiologic diagnosis in individuals through the production of specific precipitation systems. Hemoagglutination, immunofluorescence, and immunoenzymatic assay (ELISA) have application in the screening of cases as well as in the epidemiologic study of the disease. The presence of IgE antibodies and circulating antigens through radioimmunoassay procedures appear to be valuable diagnostic instruments and is presently in the course of evaluation.

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Maggots in Crime Detection

Chief Inspector Greeno arrived and organized a search for the murder weapon and shoes, coat and handbag. The masses of blowfly larvae made it difficult for us to find out much more at the time. I could see there was some kind of wound in the right forearm, but the maggots obscured it. It would need a day or two in a Lysol bath to kill them off, and we probably had a week's work in the lab ahead of us, piecing the shattered skull together. I proposed to Gardner that we should ask the Coroner, Dr. Wills Taylor, to let us take the body to Guy's to finish the job.

The Coroner agreed, and we carefully rolled the disintegrating body into the waterproof sheet. Maggots seethed out of the chest and abdominal cavity when the body was moved, and by tea-time thousands more were struggling for life in a carbolic bath in the Guy's Hospital mortuary—*Simpson K. Forty years of murder. London:Granada, 1978:72.*